

Applicant : Marc Pignot et al.
Serial No. : 09/744,641
Filed : January 26, 2001
Page : 4

Attorney's Dock : 11413-003001 / B 2286 US

REMARKS

Applicants hereby submit that the enclosures fulfill the requirements under 37 C.F.R. §1.821-1.825. The amendments in the specification merely insert the paper copy of the Sequence Listing and sequence identifiers in the specification. No new matter has been added.

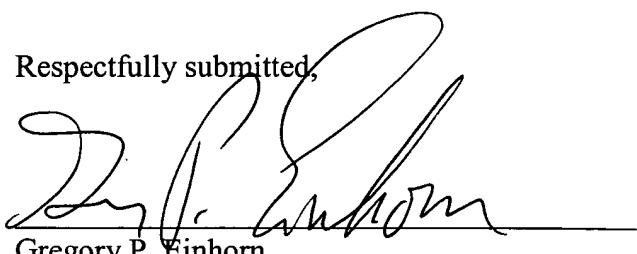
Attached hereto is a marked-up version of the changes made to the specification by the current amendment.

Please apply any charges or credits to Deposit Account No. 06-1050.

Date:

Oct. 22, 2001

Respectfully submitted,



Gregory P. Einhorn
Reg. No. 38,440

Fish & Richardson P.C.
4350 La Jolla Village Drive, Suite 500
San Diego, CA 92122
Telephone: (858) 678-5070
Facsimile: (858) 678-5099

“Version With Markings to Show Changes Made”

In the specification:

Paragraph beginning at page 9, line 1, has been amended as follows:

In Reaction Scheme 4, lower, the modification of a short duplex oligodeoxynucleotide (3•4), consisting of a plus strand oligodeoxynucleotide (5'-GCCGCTCGATGCCG-3', 3; SEQ ID NO:1) and a complementary minus strand oligodeoxynucleotide (5'-CGGCATCGA^{Me} GCGGC-3', 4; SEQ ID NO:2) with the protonated cofactor analogue 2 containing aziridine by the use of the adenine-specific DNA methyltransferase from *Thermus aquaticus* (M•*TaqI*) is shown. The complementary minus strand oligodeoxynucleotide 4 was chosen to contain N6-methyladenine-1-β-D-2'-deoxynucleoside (A^{Me}), which can not be further methylated by M•*TaqI*. M•*TaqI* usually catalyzes methyl group transfer from the natural cofactor 1 to the exocyclic amino group of adenine within the double-stranded 5'-TCGA-3' DNA sequence (Scheme 4, upper) (M. McClelland, *Nucleic Acids Res.* 1981, 9, 6795-6804).

Paragraph beginning at page 11, line 17, has been amended as follows:

The present invention, however, is not restricted to M•*TaqI* but the C5-cytosine-specific DNA methyltransferase *Haemophilus haemolyticus* (M•*HhaI*) and other methyltransferases normally using S-adenosyl-L-methionine (SAM) as cofactor can also be used. This is readily demonstrated by the modification of the duplex oligodeoxynucleotide 6•7 using M•*HhaI* (the sense and antisense strands depicted in Scheme 5 are SEQ ID NOs:3 and 4, respectively). Naturally, M•*HhaI* catalyzes the transfer of the activated methyl from SAM to the carbon atom at the 5 position of the first cytosine within the double stranded 5'-GCGC-3' DNA sequence (Scheme 5, upper). Experimental results prove that M•*HhaI* also accepts the new cofactor 2 and catalyzes its coupling to the duplex oligodeoxynucleotide 6•7 (Scheme 5, lower). Like the M•*TaqI*-catalyzed reaction, the M•*HhaI*-catalyzed coupling is quantitative.

Paragraph beginning at page 11, line 17, has been amended as follows:

The M•TaqI-catalyzed coupling of the new fluorescent cofactor 9 with the duplex oligodeoxynucleotide 3•4 (Scheme 7; sense and antisense strands are SEQ ID NOs:1 and 2, respectively) was followed by anion exchange chromatography. After proteolytic fragmentation of the formed M•TaqI-DNA complex the fluorescently labeled duplex oligodeoxynucleotide 14•4 is formed. The structure of the product 14•4 was verified by enzymatic fragmentation followed by reversed-phase HPLC. The analysis revealed besides the natural nucleosides dC, dA, dG, T, and dA^{Me} an additional fluorescent compound, which eluted with a much higher retention time than the natural nucleosides demonstrating its hydrophobic nature. This additional fluorescent compound was isolated and detected as positively charged ion at *m/z* 863.1 by electrospray ionization mass spectrometry. The observed mass is in good agreement with the calculated molecular mass of 863.4 for a protonated, with 9 modified 2'-deoxyadenosine. Thus, the coupling reaction of the new fluorescent cofactor 9 with DNA catalyzed by M•TaqI is quantitative and base-specific.